Check for updates

Original Article

Vascular

Vascular
2023, Vol. 31(1) 189–195
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/17085381211053695
journals.sagepub.com/home/vas
SAGE

COVID-19 induced aorto duodenal fistula following evar in the so called "negative" patient

Luca Traina[1], Marianna Mucignat[1,2], Roberta Rizzo[3], Roberta Gafà[2], Daria Bortolotti[3], Angelina Passaro[2] and Paolo Zamboni[2]

Abstract

Objectives: Since October 2019, SARS-CoV-2 pandemic represents a challenge for the international healthcare system and for the treatment and survival of patients. We normally focus on symptomatic patients, and symptoms can range from the respiratory to the gastrointestinal system. In addition, we consider patients without fever and respiratory symptoms, with both a negative RT nasopharyngeal swab and lung CT, as a "Covid-19 negative patient." In this article, we present a so called Covid-19 "negative" patient, with an unsuspected vascular clinical onset of the viral infection.

Methods: An 80 y.o. man, who previously underwent endovascular aortic repair for an infrarenal abdominal aortic aneurysm, presented to our department with an atypical presentation of an aorto-enteric fistula during the pandemic. While in hospital, weekly nasopharyngeal swab tests were always negative for SARS-CoV-2. However, the absence of aortic endograft complications, the gross anatomy of duodenal ischemic injury, and the recent history of the patient who lived the last months in Bergamo, the Italian city with the highest number of COVID-19 deaths, lead the senior Author to suspect an occult SARS-CoV-2 infection. The patient underwent to resection of the fourth portion of the duodenum and the first jejunal loop, with subsequent duodenum–jejunal latero-lateral anastomosis and the direct suture of the aortic wall. The intestinal specimen was investigated as suspected SARS-CoV-2 bowel infection by the means of immune-histochemistry (IHC). An ileum sample obtained in the pre-COVID-19 era was used as a control tissue.

Results: The histological analysis of the bowel revealed sustained wall ischemia and liponecrosis of the duodenal wall, with intramural blood vessels thrombosis. Blood vessel endotheliitis and neo-angiogenesis were also observed. Finally, the IHC was strongly positive for SARS-CoV-2 RNA and for HLA-G presence, with a particular concentration both in blood vessels and in the intestinal villi. The control tissue sample was not positive for both SARS-CoV-2 and HLA-G.

Conclusions: Coronavirus pandemic continues to be an international challenge and more studies and trials must be done to learn its pathogenesis and its complications. As for thromboembolic events caused by SARS-COV-2, vascular surgeons are involved in treatment and prevention of the complications of this syndrome and must be ready with general surgeons to investigate atypical and particular cases such as the one discussed in this article.

Keywords

COVID-19, aorto-enteric fistula, endovascular aortic repair, HLA-G, SARS-CoV-2, bowel ischemia

Introduction

Aorto-enteric Fistula (AEF) is a rare but life-threatening complication following aortic aneurism repair. The incidence of AEF is different between open (up to 1.6%)[1,2] and endovascular approach (up to 0.8%).[3] The latter can be associated with endoleak, whereas aortic wall inflammation or primary graft infection were both more frequently reported as late complications of open repair. However, AEF can lead itself to a secondary graft infection.[4,5] The therapy

[1]Unit of Vascular and Endovascular Surgery, Azienda Ospedaliero Universitaria di Ferrara – Arcispedale S.Anna, Ferrara, Italy
[2]Department of Translational Medicine for Romagna, and Vascular Diseases Center, University of Ferrara, Ferrara, Italy
[3]Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Corresponding author:
Marianna Mucignat, Unit of Vascular and Endovascular Surgery, Azienda Ospedaliero Universitaria di Ferrara – Arcispedale S.Anna, via Aldo Moro, 8, 44124 Cona, Ferrara (FE), Italy.
Email: dr.ssa.mucignat@gmail.com

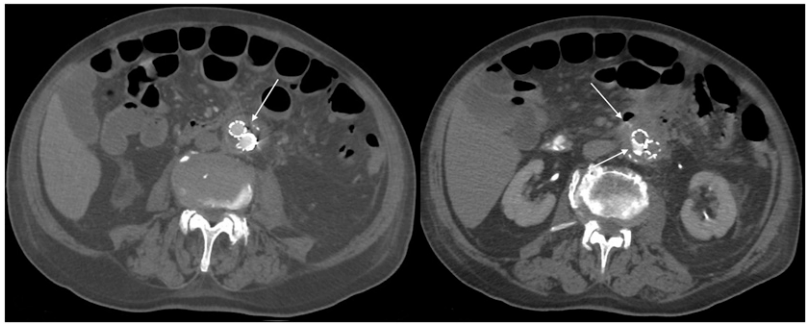

Figure 1. Left. CT scan with endovenous contrast: no visible signs of endoleak. The arrow indicates bubbles in the aortic aneurysm sac. Right. CT scan with oral contrast. The arrows indicate contrast in the aortic aneurysm sac.

for this event is variable, depending on the surgeon experience and patient clinical conditions.[4,6]

COVID-19, a viral infection initially thought to be prominently an interstitial pneumonia,[7,8] can be considered as a vascular disease with regards to serious complications and mortality. Quite recently, blood clots have emerged as the common factor unifying many of the symptoms currently without an apparent explanation.[9]

In this article, we present a case of AEF years after endovascular aortic repair (EVAR) procedure during COVID-19 pandemic era.

Materials and methods

An 80 y.o. male presented to the emergency department for persistent hyperpyrexia, abdominal pain, and asthenia. The patient came from Bergamo, a city located in northern Italy and, at the time of his presentation at the emergency department, the city was considered as a high-risk zone for coronavirus infection. A RT nasopharyngeal swab for SARS-COV-2 was performed, with negative result. The thoracic CT was unremarkably confirming the molecular data and the absence of classic symptomatology of Covid-19 disease, as well.

The clinical history was positive for previous myocardial infarction (treated with PTCA) with evolution in dilatative chronic ischemic heart disease and apical left ventricle aneurysm, dyslipidemia, and polyneuropathy with demyelination. In February 2019, the patient underwent EVAR procedure for abdominal aortic aneurysm. Laboratory exams were performed with finding of neutrophilic leukocytosis and elevation of reactive C-protein.

A CT scan of the abdomen was performed, revealing the dilation of the aortic aneurysm sac without frank images of type II endoleak. In the cranial portion of the sac, there was presence of some fluid collections with bubbles. Furthermore, the sac appeared to be in tight and indissociably adherent to the duodenum wall.

At first, a conservative approach was considered with the consultant in infective disease, starting an antibiotic therapy with piperacillin/tazobactam and daptomycin, and monitoring the patient with a new CT after 48 h of therapy.

The new CT showed new fluid lining in the liver and in the rectovesical pouch, associated with levels in the small intestine. After the ingestion of hydrosoluble contrast, an aorto-duodenal fistula was clearly detected (Figure 1).

At laparotomy, ischemic necrosis of the distal fourth of the duodenum was found, which appeared connected to the aneurism cavity via fistulae. The aortic prosthesis was undamaged, and there was no endo-leakage. In agreement with vascular and general surgeons, the patient underwent to drainage of the periprosthetic collection, resection of the fourth portion of the duodenum and the first jejunal loop, with subsequent duodenum–jejunal latero-lateral anastomosis (Figure 2). The aortic wall tear was fixed with a direct suture and an omentopexy.

During hospital stay, the patient underwent various antibiotic therapies, narrowed down based on the microbiological results of the fluid abdominal collections taken in the operatory theater and lining with clinics and laboratory findings of the patient. Several controls with abdominal CT scan were made, showing at first a new capsulated fluid collection in the upper segment of the abdominal aorta with inflammation of the aortic wall. After 17 days of antibiotic therapy, a new CT scan showed the complete disappearance of fluid collections, correlating with the better clinical condition of the patient.

While in hospital, weekly tests were always negative for SARS-CoV-2. However, the absence of aortic endograft complications, the gross anatomy of duodenal ischemic injury, and the recent history of the patient who lived the last months in Bergamo, the Italian city with the highest number of COVID-19 deaths, led the Senior Author to hypothesize a hiding SARS-CoV-2 infection. The Ethical Committee approved the investigation of suspected SARS-CoV-2 specimen by the means of immune-histochemistry (IHC).

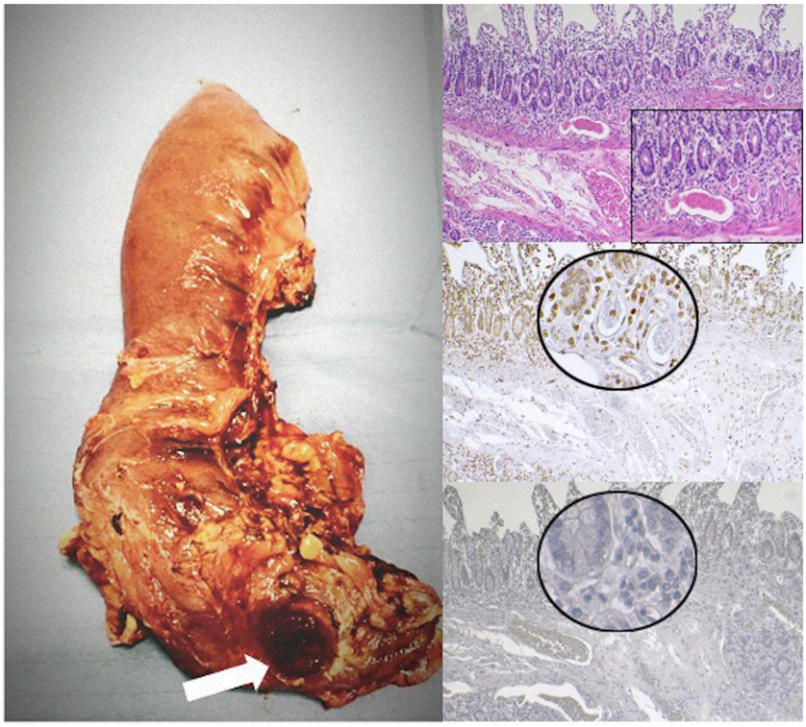

Figure 2. Left. Specimen of the resected fourth segment of the duodenum and first jejunal loop with evident ischemic injury. The arrow indicates the fistula in the posterior aspect of the duodenal wall. Right Optical Microscopy. Top panel: Hematoxylin-eosin 10×. Patchy ischemia of the duodenum with surface necrosis and fibrin thrombi; inset 20×: inflammatory infiltration and fibrin thrombi. Mid and bottom panel: Positive staining respectively for SARS-CoV-2 virus and HLA-G, IHC 10×; inset 40× mid and bottom panel: areas with strong IHC positivity.

Three months after discharge, CT showed that the aortic graft continued to function well.

Histology and IHC

The resected intestine was fixed in formaldehyde and embedded in paraffin. As control tissue, an ileum sample obtained in the pre-COVID-19 era was used.

For histological examination, slides were stained with hematoxylin, and eosin. IHC analysis was performed on 4-µm thick sections: following antibody retrieval (heating), the slides were treated with anti-SARS-CoV-2 nucleocapsid protein (Novus Biologicals, Centennial, 1:250 dilution) or anti-human HLA-G (MEM-G2, Exbio, dilution 1:200). Positivity was detected by horseradish peroxidase/3,3′-diaminobenzidine.

Ethics approval

The study was approved by our hospital's ethics committee (Number: 540/2020/Oss/AOUFe-20/05/2020). All the data were anonymized, and no connection with the patient identity was possible. A consent form was signed by the patient.

Results

Macroscopically, the resected bowel specimen showed signs of ischemic suffering such as the beginning of tissue necrosis and some petechial hemorrhages. In the posterior aspect of the duodenal wall, the presence of an ulcer was detected, identifying the duodenal fistula (Figure 2).

The histological analysis of the bowel revealed sustained wall ischemia and liponecrosis of the duodenal wall, with intramural blood vessels thrombosis (Figure 2). Blood vessel endotheliitis and neo-angiogenesis were also observed. Finally, the IHC was strongly positive for SARS-CoV-2 RNA and for HLA-G presence, with a particular concentration either in blood vessels and in the intestinal villi. The control tissue sample was not positive for both SARS-CoV-2 and HLA-G.

Discussion

Secondary AEF is a rare and life-threatening complication of both open and endovascular aortic repair, with a significant range of morbidity and mortality, ranging between 14% and 75%.[10,11]

Considering open repair, we can recognize two types of secondary AEF, based on the site of the communication

between the aorta (or the surgical graft) and the bowel. The first type is defined as graft enteric fistula (GEF), and it occurs when the communication is located between the graft-aorta anastomotic site and represents the most common type of AEF. The second type is defined as graft enteric erosion (GEE), and it occurs with a breakdown of the enteric wall overlying the graft resulting in bathing of the graft by enteric contents and chronic graft infection.[12]

If we consider the endovascular approach, AEF can be associated with endoleak, aortic wall inflammation, stent migration, kinking, and graft infection.[1,3,13] The incidence of AEF seems to be higher in inflammatory abdominal aortic aneurysm than in the atherosclerotic one, despite the higher incidence of rupture in the latter.[14]

Clinical presentation is usually with gastrointestinal bleeding with hematemesis, melena[1] and fecal occult blood, depending on the location of the fistula. Literature describes other more insidious and less frequent presentations such as sepsis, high fever, diffuse abdominal pain, endocarditis,[15] and septic arthritis.[13]

Diagnosis of AEF can be insidious, especially if symptoms include fever and sepsis. In fact, CT scan reveals AEF up to 80% of cases[16] and a clear disruption of the aortic wall with contrast enhancement in the duodenum cannot always be seen. Moreover, endoscopy is rarely performed if the patient shows no signs of gastrointestinal bleeding, and when performed, it does not exclude diagnosis if no fistula is seen.[17]

The management of this rare complication is not well defined and is tightly connected with age and comorbidities of the patient. Surgical strategies include extra-anatomical bypass with aortic ligation and graft removal, in situ aortic reconstruction, endovascular aortic repair,[10,17] and aortic direct suture. However, the choice of the technique of revascularization in AEF is controversial: extra-anatomical bypass is usually chosen when the graft or surrounding tissue is highly contaminated with a purulent fluid collection or gross retroperitoneal infection. Surgical intervention for enteric fistula is an integral part in both in situ reconstruction and extra-anatomical bypass. Simple closure of the enteric fistula may be performed when the defect is small. If the defect is large, segmental bowel resection should be performed to prevent leakage resulting in recurrent infection.[18] Omentopexy can also be a surgical option if the aortic disruption is small.[18]

Each surgical technique must be associated with endovenous antibiotic therapy and optimization of the nutritional status of the patient.[10]

Since October 2019, SARS-CoV-2 pandemic represents a real challenge for healthcare system and for the understanding of its many clinical presentations and pathogenesis.

By now, it is known that coronaviruses have on their surface Spike glycoproteins,[19,20] which represent the most immunogenic parts of the virus, and may bind to angiotensin-converting enzyme-2 (ACE-2) receptors to enter the host cell.[21,22]

ACE-2 receptors expression has been found on the surface of alveolar epithelial type II cells, cardiac, renal, intestinal, and endothelial cells and is consistent with the target organs involved and the clinical picture in COVID-19 infection.[22–25]

Despite the suspect of finding coronavirus at the level of the duodenum, we were of course surprised to find the evidence of the viral infection in a so called “negative” patient. Our finding suggests that a patient could have an asymptomatic/oligosymptomatic classic respiratory COVID-19 disease followed some months later by a vascular/intestinal complication. To confirm our clinical conclusion, we also performed an IHC in a control tissue in order to be sure of the viral detection. There are other cases of late intestinal complications following a successful treatment of COVID-19 presenting as atypical ulceration and bleeding, where immunohistochemical analysis of the intestinal specimen with anti–SARS-CoV-2 nucleocapsid protein revealed the presence of viral protein expression in epithelial cells of the mucosa and in a minority of lymphocytes.[26]

Studies may suggest that coronavirus, after entering the host cell, unleashes the macrophage activation syndrome and a massive cytokine storm,[19,24] with the particular release of IL-10: this cytokine seems to upregulate the expression of HLA-G on the surface of the infected cells.[25,27–29] The expression of such non-classical human leukocyte antigen, found in the same areas where IHC detected SARS-CoV-2 as well as inflammatory and ischemic injury, is an original finding. Speculatively, we may hypothesize that it is an intriguing piece of the pathogenesis of the duodenal ischemia. We know that HLA-G has the capability to downregulate the immune response, inhibiting T cells, NK cells, and B cells, and causing cell death and tissue necrosis.[25,27,28,30–32,33] HLA-G gene is characterized by polymorphisms at the 3′ un-translated region and 5′ upstream regulatory region that increases viral infection susceptibility, creating an unbalanced and pathologic environment.[29] HLA-G up-regulation is proposed to be associated with virus-encoded homologs of human IL-10 (cmvIL-10),[30] which prevents NK cell recognition of infected cells, an extraordinary mimetic mechanism with a possible role in the case herein described. The above effect may contribute to reduce the efficiency of our immune system thus permitting a more florid viral replication at the level of the intestinal vessels. Furthermore, this hypothesis is corroborated by an investigation at the level of the coronary artery disease which concluded that non-classical HLA class I molecules are capable of inducing an immune tolerant microenvironment.[34]

To support the role of coronavirus as a primary agent in gastrointestinal symptoms, SARS-CoV-2 RNA has been

found in stool specimens in association with high levels of fecal calprotectin, as to confirm inflammation of the bowel system.[24,35,36]

This singular case shows no presence of endoleak, endotension, or primary infection of the endovascular graft, so there does not seem to be any prosthesis implication in the pathogenesis of the AEF for this patient. Considering the discussion above, despite the negativity of the nasopharyngeal swab and chest CT, our findings on the analyzed bowel piece and the geographical origin of the patient lead us to hypothesize that the subject was previously stricken by an asymptomatic Covid-19 infection. To explain our findings, we hypothesize that subsequently the virus settled in the intestine causing such an atypical and dramatic gastrointestinal complication.

It is estimated that at least more than 80% of persons infected with the SARS-CoV-2 virus have an asymptomatic course. Practically, almost nothing is known about the asymptomatic cases, since our knowledge of COVID-19 is limited to hospitalized.[37,38]

Some may argue that it would be unlikely that a virus, whose typical manifestations are mainly respiratory, should primarily affect the gut. On the other hand, we know that, following a COVID-19 interstitial pneumonia, the SARS-CoV-2 virus may persist longer in the gastrointestinal tract. For instance, viral RNA was detected in the feces in 70.3% of Covid-19 patients (95% CI, 49.6–85.1) after oro/naso pharyngeal swab had become negative.[37,39]

We are unaware why the hypercoagulable status induced by the virus led to extensive thrombosis of the parietal duodenum vessels, as well as if the mechanical pulsation of the repaired aortic aneurism cavity triggered it in some way. These are still open questions. However, the association of positive staining for HLA-G in association with SARS-CoV-2 warrants further investigations in order to understand the possible role of the former as pro-thrombotic inductor.[20,34,40]

Conclusions

As we can see today, coronavirus pandemic continues to be an international challenge and more studies and trials must be done to learn its pathogenesis and its complications. As for thromboembolic events caused by SARS-CoV-2, vascular surgeons are involved in treatment and prevention of the complications of this syndrome and must be ready with general surgeons to investigate atypical and particular cases such as the one discussed in this article.

Author Contributions

All the authors contributed to the design and conception of this studyRizzo, Gafà, Bortolotti, Passaro, and Zamboni contributed to collection, analysis, and interpretation of the dataPassaro and Zamboni obtained fund for this study from the crowdfunding "Il Covid ha mille facce. Combattiamole tutte." (https://crowdfunding.unifeel.it/progetto/il-covid-ha-mille-facce-combattiamole-tutte/) devoted to COVID-19 research of the University of Ferrara (institutional funds)Mucignat and Zamboni assume overall responsibility for this work.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The research has been carried out with our own Institutional Funds

ORCID iD

Marianna Mucignat https://orcid.org/0000-0002-2741-9499

References

1. Arworn S, Orrapin S, Chakrabandhu B, et al. Aorto-enteric fistula after endovascular abdominal aortic aneurysm repair for Behcet's disease patient: a case report. *EJVES Short Rep* 2018; 39: 54–57
2. Dorigo W, Pulli R, Azas L, et al. Early and long-term results of conventional surgical treatment of secondary aorto-enteric fistula. *Eur J Vasc Endovasc Surg* 2003; 26: 512–518.
3. Kahlberg A, Rinaldi E, Piffaretti G. et al. MAEFISTO collaborators, Results from the multicenter study on aortoenteric fistulization after stent grafting of the abdominal aorta (MAEFISTO). *J Vasc Surg* 2016; 64: 313e20.
4. Niaz OS, Rao A, Abidia A, et al. Surgical and medical interventions for abdominal aortic graft infections. *Cochrane Database Syst Rev* 2020; 8: CD013469. doi: 10.1002/14651858.CD013469.pub2
5. OTA Lyons, Baguneid M and Barwick TD. Diagnosis of aortic graft infection: a case definition by the Management of Aortic Graft Infection Collaboration (MAGIC). *Eur J Vasc Endovas Surg* 2016; 52(6): 758–763.
6. Fitzgerald S, Kelly C and Humphreys H. Diagnosis and treatment of prosthetic aortic graft infections: confusion and inconsistency in the absence of evidence or consensus. *J Antimicrob Chemother* 2005; 56: 996–999
7. Prompetchara E, Ketloy C and Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. *Pac J Allergy Immunol* 2020; 38(1): 1–9. doi: 10.12932/AP-200220-0772.
8. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; 323: 1061.
9. Zamboni P. COVID-19 as a vascular disease: lesson learned from imaging and lood biomarkers. *Diagnostics (Basel)* 2020; 10(7): 440. doi: 10.3390/diagnostics10070440

10. Chopra A, Cieciura L, Modrall JG, et al. Twenty-year experience with aorto-enteric fistula repair: gastrointestinal complications predict mortality. *J Am Coll Surg* 2017; 225(1): 9–18.
11. Vogel T, Symons R and Flum D. The incidence and factors associated with graft infection after aortic aneurysm repair. *J Vasc Surg* 2008; 47(2): 264–269.
12. Pipinos II, Carr JA, Haithcock BE, et al. Secondary aortoenteric fistula. *Ann Vasc Surg* 2000; 14: 688–696.
13. Parry DJ, Waterworth A, Kessel D, et al. Endovascular repair of an inflammatory abdominal aortic aneurysm complicated by aortoduodenal fistulation with an unusual presentation. *J Vasc Surg* 2001; 33: 874–879.
14. Zaki M, Tawfick W, Alawy M, et al. Secondary aortoduodenal fistula following endovascular repair of inflammatory abdominal aortic aneurysm due to Streptococcus anginosus infection: a case report and literature review. *Int J Surg Case Rep* 2014; 5(10): 710–713. doi: 10.1016/j.ijscr.2013.10.016. Epub 2014 Aug 12.
15. Kadhim MMK, Rasmussen JBG and Eiberg JP. Aorto-enteric fistula 15 years after uncomplicated endovascular aortic repair with unforeseen onset of endocarditis. *EJVES Short Rep* 2016; 31: 16–18.
16. Chenu C, Marcheix B, Barcelo C, et al. Aorto-enteric fistula after endovascular abdominal aortic aneurysm repair: case report and review. *Eur J Vasc Endovasc Surg* 2009; 37: 401–406.
17. O'Connor S, Andrew P, Batt M, et al. A systematic review and meta-analysis of treatments for aortic graft infection. *J Vasc Surg* 2006; 44(1): 38–45.
18. Koda Y, Murakami H, Yoshida M, et al. Secondary aortoenteric fistula and type II endoleak five years after endovascular abdominal aortic aneurysm repair. *EJVES Short Rep* 2019; 43: 12–17.
19. Robson B. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance. *Comput Biol Med* 2020; 121: 103749. doi: 10.1016/j.compbiomed.2020.103749. Epub 2020 Apr 11.
20. Bortolotti D, Gentili V, Rizzo S, et al. SARS-CoV-2 spike 1 protein controls natural killer cell activation via the HLA-E/NKG2A pathway. *Cells* 2020; 9(9): 1975. doi: 10.3390/cells9091975
21. Soy M, Keser G, Atagündüz P, et al. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol* 2020; 39(7): 2085–2094. doi: 10.1007/s10067-020-05190-5. Epub 2020 May 30.
22. Gu J, Han B and Wang J. COVID-19: gastrointestinal manifestations and potential fecal-oral transmission. *Gastroenterology* 2020; 158: 1518–1519.
23. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol* 2020; 203: 622–630. doi: 10.1002/path.1560
24. Ojetti V, Saviano A, Covino M, et al. GEMELLI AGAINST COVID-19 group, COVID-19 and intestinal inflammation: role of fecal calprotectin. *Dig Liver Dis* 2020; 52(11): 1231–1233. doi: 10.1016/j.dld.2020.09.015. Epub 2020 Sep 22.
25. Zidi I. Puzzling out the COVID-19: therapy targeting HLA-G and HLA-E. *Hum Immunol* 2020; 81(12): 697–701. doi: 10.1016/j.humimm.2020.10.001. Epub 2020 Oct7.
26. Rizzo R, Neri LM, Simioni C, et al. SARS-CoV-2 nucleocapsid protein and ultrastructural modifications in small bowel of a 4-week-negative COVID-19 patient. *Clin Microbiol Infect* 2021; 27(6): 936–937. doi: 10.1016/j.cmi.2021.01.012. Epub 2021 Jan 16.
27. Zhang S, Gan J, Chen BG, et al. Dynamics of peripheral immune cells and their HLA-G and receptor expressions in a patient suffering from critical COVID-19 pneumonia to convalescence. *Clin Transl Immunol* 2020; 9(5): e1128. doi: 10.1002/cti2.1128. eCollection 2020 May.
28. Urosevic M, Kurrer MO, Kamarashev J, et al. Human leukocyte antigen G up-regulation in lung cancer associates with high-grade histology, human leukocyte antigen class I loss and interleukin-10 production. *Am J Pathol* 2001; 159: 817.
29. Rizzo R, Bortolotti D, Bolzani S, et al. HLA-G molecules in autoimmune diseases and infections. *Front Immunol* 2014; 5: 592. doi: 10.3389/fimmu.2014.00592
30. Spencer JV, Lockridge KM, Barry PA, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol* 2002; 76: 1285–1292. doi: 10.1128/JVI.76.3.1285-1292.2002
31. Li C, Toth I, Schulze Zur Wiesch J, et al. Functional characterization of HLA-G+ regulatory T cells in HIV-1 infection. *PLoS Pathog* 2013; 9: e1003140.
32. Yan WH, Lin A, Chen BG, et al. Induction of both membrane-bound and soluble HLA-G expression in active human cytomegalovirus infection. *J Infect Dis* 2009; 200: 820–826.
33. Amiot L, Vu N, Rauch M, et al. Expression of HLA-G by mast cells is associated with hepatitis C virus-induced liver fibrosis. *J Hepatol* 2014; 60: 245–252.
34. Zidi I, Kharrat N, Abdelhedi R, et al. Nonclassical human leukocyte antigen (HLA-G, HLA-E, and HLA-F) in coronary artery disease. *Hum Immunol* 2016; 77(4): 325–329. doi: 10.1016/j.humimm.2016.01.008. Epub 2016 Jan 11.
35. Wong MCs, Huang J, Lai C, et al. Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis. *J Infect* 2020; 81(2): e31–e38. doi: 10.1016/j.jinf.2020.06.012. Epub 2020 Jun 11.
36. Ayling RM and Kok K. Fecal calprotectin. *Adv Clin Chem* 2018; 87: 161–190.
37. Wang Y, Wang Y, Chen Y, et al. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus

pneumonia (COVID-19) implicate special control measures. *J Med Virol* 2020; 92: 568–576. doi: 10.1002/jmv.25748.

38. Wu Z and McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (covid-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323: 1239–1242. doi: 10.1001/jama.2020.2648
39. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; 5: 434–435.
40. Rizzo R, Neri LM, Simioni C, et al. SARS-CoV-2 nucleocapsid protein and ultrastructural modifications in small bowel of a 4-week-negative COVID-19 patient. *Clin Microbiol Infect* 2021. doi: 10.1016/j.cmi.2021.01.012.